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Developmental responses of sterlet (*Acipenser ruthenus*) to temperature modulation with insights into intestinal and morphological traits

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Sturgeon fish are highly valued in aquaculture, relying on optimal water temperatures for health and productivity. In this study, three temperatures were considered including group A: (ambient temperature, 18 ºC), group B: 21 ºC and group C: 27 ºC to investigate the intestine morphology, growth performance, meristic and morphological traits of sterlet. For this purpose, the temperature was gradually increased, and the larvae were exposed at the desired temperature 32 days after fertilization (dpf) and then returned to ambient temperature until 64 dpf. The growth results indicated that temperature manipulation in the first two months of the life can improve growth. Examining the morphologic and meristic characteristics showed that the increase in temperature accelerates fin and skeletal development, in which the calcified structures such as lateral scutes, distal radials and spine of pectoral fin were detected in group C. The development of villi's was higher in group C than the other groups at 32 dpf and 64 dpf. Therefore, the results of this study demonstrated that short-term thermal tolerance of sterlet has a positive effect on growth performance, morphological traits and intestine development. Proper temperature control enhances sturgeon aquaculture in the first two months.

Keywords Growth performance, Gut histology, Meristic characteristics, Thermal tolerance, Acipenseridae

Sturgeons are one of the most ancient vertebrate species still existing on Earth, with a lineage dating back approximately 250 million years[1]. The growing demand for sturgeon production underscores the need to advance hatchery technologies for producing higher-performing larvae. These fish face declining natural catches, while sustained demand for their meat and caviar has led to opportunities for sturgeon farming[2]. Sterlet (*Acipenser ruthenus*, Linnaeus, 1758) as the smallest fish in sturgeon family is a model for various experiments due to its availability, manageable size and shorter maturation period[3,4]. Larval production is crucial in sturgeon cultivation, as larvae are highly susceptible to environmental fluctuations, especially during early development, impacting respiration, growth and survival[5,6].

Temperature plays a crucial role in ectotherms during incubation, embryonic stages, and larval development, influencing growth and physiology[5,7]. Warmer temperatures can speed up metabolism by 2–3 times[8]. However, if temperatures go exceed the species' tolerance, it may reduce growth rate, disrupt development, and increase mortality rate, leading to higher costs and lower production stability[9,10]. Studies have shown that the effects of temperature can vary depending on the species and the developmental stage in sturgeon fish[11,12]. Some studies focus on the period from hatching to yolk sac absorption, while have extended the observation period, demonstrating enhanced growth at elevated temperatures[11-14]. To fully understand the impact of temperature on larval development, it is important to examine the appropriate larval period, as short-term studies may not capture the full effects. For example, no growth differences were found in Siberian sturgeon (*Acipenser baerii*) larvae exposed to 16–22 ºC, while beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*) exhibited higher growth rates at 18–19 ºC compared to 24–25 ºC during the initial larval development[5,11]. No significant growth differences were observed in sterlet at 20 ºC and 25 ºC[15]. A comparable result was found for white sturgeon (*Acipenser transmontanus*), where no significant growth differences were observed between 20 ºC and 24 ºC[16].

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In contrast, better growth was observed in green sturgeon (*Acipenser medirostris*) and lake sturgeon (*Acipenser fluvescens*) at 24 °C[12,14]. These differences may be attributed to the evolutionary adaptations to native habitats, as some sturgeons are adapted to cold rivers while the others thrive in warmer waters. Since temperature plays a crucial role in every stage of development, it directly influences growth, metabolism, and overall physiology[17,18]. Therefore, species-specific and time-sensitive assessments are crucial for optimizing rearing conditions and ensuring sustainable aquaculture by accurately evaluating temperature effects on sturgeon larvae.

Since growth is a highly plastic trait, fish adjust their developmental processes in response to environmental fluctuations, with phenotypic plasticity enabling modifications in morphology, physiology, and phenotype expression[19]. Environmental changes during phenocritical periods can shape these traits, potentially leading to lasting modifications in gene expression that persist into adulthood[20]. Thus, body traits reflect both genetic and environmental factors[21]. The early ontogenic development of Siberian sturgeon and white sturgeon during embryonic and yolk sac larvae stages has been shown to be significantly influenced by increasing temperature[18,,22,,23]. While the effects of temperature on growth, developmental traits, and morphological defects are well-established in teleost fish[24-26], there is a significant gap in research on how temperature influences morphological traits and skeletal development in sturgeon larvae at more advanced developmental stages.

In sturgeons, the endoskeleton is mainly cartilage, and some areas covered by mineralized structures like scutes as exoskeleton[27]. As fish grow, their skeletons develop to support swimming, foraging, and protection. Therefore, study the skeletal system during the early stages of development can serve as a valuable tool for identifying optimal rearing conditions.

Intestine as a critical organ during the developmental stage is substantial for fish health, impacting nutrient absorption, immunity, and growth[2,28]. Given its crucial role, its activity is highly responsive to temperature variations, which can influence digestive efficiency and overall fish health[8,29]. Temperature effects on intestinal morphology vary by species, reflecting differences in biology, habitat, and acclimatization[8,30]. Studies have shown that exposure to low temperatures reduces feeding activity and impaired nutrient absorption leading to decline in intestine morphological parameters[31]. In warmwater fish, such as common carp (*Cyprinus carpio*), elevated temperatures have been associated with significant reduction in villi height in the midgut resulting in decreased absorptive capacity[32]. Conversely, in juvenile butter catfish (*Ompok bimaculatus*), the highest villi height was observed at a rearing temperature of 30 °C[33]. Most studies on sturgeon species have primarily focused on the juvenile stage[34,35], leaving a significant gap in understanding the effects of temperature on intestinal development during the larval stage. Studying intestinal histology is crucial for understanding the effects of temperature on nutrient absorption, growth, and health, providing valuable insight for aquaculture and sturgeon conservation.

Understanding the effects of temperature on growth, development, and survival is crucial for improving rearing success, evaluating growth rates, and assessing the species' adaptability to changing environmental conditions. Fishes are poikilothermic creatures, in which changes are observed in physiological due to environmental factors such as water temperature[36]. Thus, the objective of this study was to investigate how temperature manipulation affects the growth performance and skeletal development of sterlet during the larval period.

Results

Growth performance

The results showed that the growth of sterlet during the larval period varied among different thermal groups. Starting at 32 dpf, group C demonstrated a significant increase in growth ($p < 0.05$) compared to other groups. This trend continued, with group C exhibiting the highest growth at 48 and 64 dpf, while group A consistently showed the lowest growth ($p < 0.05$; Fig. 2; Table 1). At the end of the experiment, SGR_W and SGR_L were significantly higher in group C compared to the group A ($p < 0.05$; Table 1), while no significant difference was observed in fish maintained in group B ($p > 0.05$). DGR varied significantly among groups, with the highest value recorded in group C and the lowest in group A ($p < 0.05$; Table 1).

	A	B	C
Initial weight (g)	0.002 ± 0.00	0.002 ± 0.00	0.002 ± 0.00
Final weight (g)	0.76 ± 0.08^{c}	1.08 ± 0.08^{b}	1.52 ± 0.08^{a}
Initial length (mm)	11.60 ± 0.00	11.53 ± 0.00	11.68 ± 0.00
Final length (mm)	48.86 ± 1.66^{b}	54.13 ± 2.25^{ab}	55.73 ± 3.53^{a}
SGR_W(% day^{-1})	10.59 ± 0.08^{b}	11.02 ± 0.22^{ab}	11.42 ± 0.24^{a}
SGR_L(% day^{-1})	2.58 ± 0.03^{b}	2.76 ± 0.04^{ab}	2.81 ± 0.06^{a}
DGR (%)	0.58 ± 0.03^{c}	0.83 ± 0.03^{b}	1.18 ± 0.03^{a}
Head area (μm^2)	14.98 ± 0.34^{b}	14.97 ± 2.06^{b}	17.82 ± 1.65^{a}
Body area (μm^2)	37.75 ± 0.87^{b}	39.80 ± 1.30^{b}	48.06 ± 6.59^{a}

Table 1. Growth parameters and morphometric traits development of Sterlet (*Acipenser ruthenus*) larvae under different temperature conditions at 64 dpf. A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C. Mean with different letters are significantly different for each group. Data are shown as mean ± standard error at $p < 0.05$. SGR_W: specific growth rate of weight; SGR_L: specific growth rate of length; DGR: daily growth rate.

Morphological and meristic traits

The development of morphometric traits in sterlet varied across temperature groups. Larvae in group C exhibited a significantly greater total length compared to the group A ($p < 0.05$; Table 1). Head area was significantly larger in group C compared to group A and group B ($p < 0.05$; Table 1). Similarly, body area varied significantly among groups with the highest value observed in group C while groups A and B showed no significant difference ($p > 0.05$; Table 1).

The development of meristic traits in sterlet were significantly affected by temperature. The number of pectoral fin radials was significantly greater in group C, with an average of 7.60 ± 0.57 ($p < 0.05$; Fig. 3A). Additionally, pelvic and anal fin radials were significantly greater in group C than in the te other groups ($p < 0.05$; Fig. 3B, C). Similarly, larvae in group C exhibited a significantly higher number of basidorsal and basiventral ($p < 0.05$, Fig. 3D, E). Lateral scutes, which are calcified structures, were observed exclusively in group C, with no presence in groups A and B ($p < 0.05$, Fig. 3F). The development of distal radials in the pectoral fins as calcified structures varied among temperature groups. In group A, no distal radials were observed, while a slight formation of distal radials appeared in group B. In contrast, group C exhibited well-defined and calcified distal radials (Fig. 4). Additionally, pectoral spines were present exclusively in group C, with no such occurrence in groups A and B (Fig. 4).

Intestine histology analysis

The intestinal morphology of sterlet was examined at 32 dpf across different rearing temperature regimes. Significant variations in tubular muscularis thickness were noted among the groups ($p < 0.05$; Table 2), with group C showing a markedly thicker muscularis layer compared to groups A and B ($p < 0.05$; Fig. 2). Villus height was also significantly influenced by rearing temperature, with group C exhibiting the tallest villi ($p < 0.05$). However, no significant differences were found in villus width or enterocyte height among the groups ($p > 0.05$; Table 2). Additionally, the intestinal perimeter varied significantly among the groups, with group C and group A showing the largest and smallest perimeter (Fig. 5A, B, C; Table 2).

At 64 dpf, the thickness of the tubular muscularis differed significantly among the groups ($p < 0.05$; Table 2), with group C displaying a considerably thicker layer compared to other groups. Similarly, group C exhibited the greatest villus height, followed by group B, while group A had the shortest villi. No significant differences in villus width or enterocyte height were observed among the groups ($p > 0.05$). Significant differences were also noted in intestinal perimeter ($p < 0.05$), with group C showing the largest perimeter (4051.10 ± 86.3 µm) and group A having the smallest (3152.40 ± 62.20 µm) (Fig. 5D, E, F; Table 3).

Discussion

Animals must develop adaptations to cope with environmental changes which are essential for their growth and survival. Temperature, as a crucial environmental factor, play a complex influence in various aspects of fish biology, including reproduction, physiology, metabolism, ontogeny, phenotype, and overall development[42,43]. This study examined the effects of temperature manipulation on intestine morphology, growth performance and morphological trait development in sterlet until 64 dpf. The gradual increase in water temperature during the first month of life could improve intestine and traits development, positively affecting growth performance in later stages.

After 64 days of rearing under optimal temperature manipulations and maintained water quality, fish exposed to elevated temperatures (group B and C) showed a significant improvement in growth performance. Notably, fish in group C, exposed to 27 °C for just 16 days, exhibited a dramatic increase in growth. Similarly, even a moderate temperature increase for fish in group B enhanced growth performance during the controlled period. Improved growth in sterlet larvae is likely due to their thermal tolerance during this critical phase,

	A	B	C
32 dpf			
Tubular muscularis (µm)	11.87 ± 0.39^{b}	12.37 ± 0.60^{a}	32.01 ± 2.54^{a}
Villi height (µm)	77.81 ± 2.61^{b}	83.64 ± 7.50^{ab}	110.38 ± 2.58^{a}
Villi width (µm)	45.04 ± 7.10	45.16 ± 3.25	63.37 ± 2.64
Enterocytes height (µm)	11.19 ± 2.65	14.35 ± 0.82	17.69 ± 2.62
Intestine perimeter (µm)	1015.10 ± 53.60^{b}	1147.62 ± 23.26^{ab}	1571.24 ± 34.76^{a}
64 dpf			
Tubular muscularis (µm)	64.39 ± 13.43^{b}	64.01 ± 6.34^{b}	205.37 ± 21.82^{a}
Villi height (µm)	261.51 ± 27.35^{b}	305.2 ± 4.13^{ab}	355.25 ± 14.43^{a}
Villi width (µm)	128.79 ± 24.03	154.28 ± 13.85	175.15 ± 11.09
Enterocytes height (µm)	68.46 ± 10.42	56.58 ± 6.37	76.21 ± 0.67
Intestine perimeter (µm)	3152.40 ± 62.20^{b}	3250.02 ± 30.03^{b}	4051.10 ± 86.3^{a}

Table 2. Intestinal histological parameters of Sterlet (*Acipenser ruthenus*) larvae under different rearing temperature regimes. A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C. Mean with different letter are significantly different for each group. Data are shown as mean ± standard error (SE) at $p < 0.05$.

which enhances metabolism, food intake, digestion, and nutrient absorption. As a result, more energy is directed toward growth[44].

In the intestine level, enhanced growth in fish exposed to high temperatures is associated with improved intestinal function, as digestion and nutrient absorption rely on the integrity of the villi and digestive surface[45]. Intestinal perimeter, villi height, villi width, and muscularis thickness are key indicators for assessing intestinal function[46]. Sterlet larvae in group C demonstrated greater values for tubular muscularis thickness, villi height, and intestine perimeter. The thicker muscularis suggests that higher temperatures promote intestinal wall development, improving digestive efficiency[47-49]. This is consistent with findings in other sturgeon species, where a fully developed digestive system supports efficient feeding on artificial diets[38,50]. The increased villi height in group C at both time points (32 and 64 dpf) reflects enhanced structural capacity for nutrient absorption. These results align with studies on stellate sturgeon (*Acipenser stellatus*) and Siberian sturgeon[51,52]. The observed increase in intestinal perimeter in group C may indicate an expansion of the intestinal lumen, providing more surface area for nutrient absorption. These findings are consistent with prior studies that report a reduction in intestinal fold height and an increase in muscularis thickness as fish adapt to nutrient-dense diets[51,53]. Generally, short-term exposure to higher temperatures during appropriate larval stages helps the digestive system develop, allowing better adaptation to artificial diets. However, potential trade-offs, including increased energy demands and the risk of disrupting intestinal balance, need to be considered, with examining the impact of temperature on digestive enzymes.

Studies on sturgeon growth have reported inconsistent findings regarding this effect. In Siberian sturgeon, the optimal growth was observed at 18 °C, while green sturgeon and lake sturgeon exhibited enhanced growth with rising temperatures, reaching up to 24 °C[5,12,14]. Similarly, studies on beluga and Persian sturgeon have reported optimal growth at 18 and 19 °C, respectively[11]. These suggests that the impact of temperature changes is influenced by the species, the temperature range, and the specific life stage at which the larvae are exposed[54]. Fish experience different life stages, each with varying responses to temperature changes, with the larval stages from embryo to hatching being especially sensitive[55]. Temperature was gradually increased from 8 dpf in this study, which aligns with the approach used for green sturgeon. In both cases, temperature manipulation was delayed until near yolk-sac absorption to prevent exposing newly hatched larvae to elevated temperatures[12]. Newly hatched larvae are highly sensitive to temperature fluctuations and unable to adapt fully to elevated thermal conditions, leading to reduced growth at higher temperatures, as observed in other sturgeon species[5,11]. This highlights the importance of identifying the optimal timing for temperature manipulation, as thermal tolerance in different developmental stages and species[56]. It is reported that a short exposure to elevated temperatures during embryonic and larval stages can enhance the organism's ability to tolerate higher temperatures later in life[18,57] that is in agreement with current study. The examination on sterlet have demonstrated that constant exposure to higher temperatures (20 °C and 25 °C) did not significantly affect growth[15]. This discrepancy may be attributed to the differing thermal regimes used in these studies, which involved constant temperature exposure. In contrast, the present study employed a variable temperature regime, potentially explaining the observed differences in growth responses. This phenomenon highlights the complex interplay between temperature and development in fish, emphasizing the need to consider specific thermal preferences.

Temperature is a key factor affecting external morphology during development, making it important to study environmental influences in early stages to understand phenotypic plasticity in fish[58,59]. Group C showed significantly faster development, including fin spine formation, scute development, and growth of the head and body, compared to other groups, highlighting that temperature has a significant effect on the rate of developmental progress. The increase on head size and body size observed in this study aligns with findings in white sturgeon, where traits such as head, mouth, gill filament, and pectoral fin areas expanded over time in warmer temperatures[18]. This enhanced development likely contributes to improved respiratory function and overall activity levels[60]. An increase in meristic traits, such as pectoral, pelvic, and anal fins radials, indicates greater plasticity in warmer water, likely enhancing motor control, slow movement, and stability in the water column. The influence of environmental conditions on meristic traits has been confirmed by previous studies, which align with the findings of the persent study[19,26].

In this study, the development of lateral scutes, spine calcification, and pectoral fin radials was closely linked to temperature, with larvae in group C showing more pronounced changes. This fortification can improve swimming speed during key life stages, allowing larvae to allocate energy more effectively toward growth and development. Similarly, temperature effects on growth and mineralization vary across species. For example, in brown trout (*Salmo trutta*) fry, elevated temperatures delayed the growth, resulting in lower mineralization levels but conversely, in gilthead Sea bream (*Sparus aurata*) and wolffish (*Anarhichas lupus*) higher temperatures stimulated enhanced calcification level and bone formation[60-62]. This improvement highlights the strong link between skeletal development and fish size, rather than age[19,63]. Faster development may help larvae escape predators by starting to drift earlier, thereby reducing their exposure to predation and environmental threats during the susceptible stages of development[13,18,26,64,65]. These growth characteristics reflected the acquisition of juvenile traits, suggesting a shift toward a more developed morphology. This shift may be associated with the thyroid gland's response to temperature changes, which influence development and metamorphosis[60,66]. Future studies should investigate the impact of temperature on the hypothalamus-pituitary-thyroid axis in sturgeon larvae.

In conclusion, the result of this study showed that sterlet larvae grew faster in group C during first month of life and increased morphologic and meristic traits development. Similarly, elevated temperatures promoted intestinal development reflecting enhanced nutrient absorption potential and digestive capacity. Further examinations and an extended experimental duration are necessary to determine whether the changes in growth performance, intestinal histology, and morphological development persist at later stages. This approach would also help to evaluate the prolonged effects of temperature while exploring the functional implications

Groups	Days post fertilization																								
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
A	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
B	18	19	20	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
C	18	19	20	21	22	23	24	25	26	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27

Table 3. Thermal status of the experiment in different days. All groups were returned to ambient temperature (18 °C) from 32 dpf to 64 dpf. A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C.

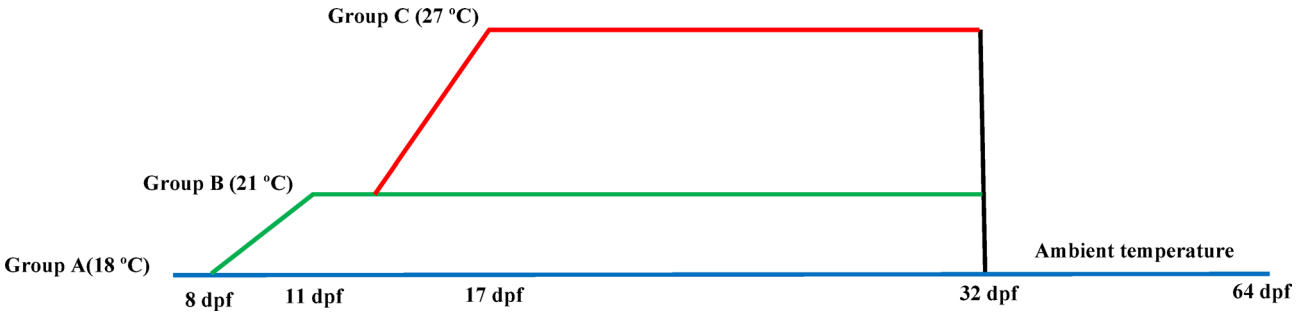

Fig. 1. Schematic view of different experimental groups under temperature manipulation during the 64-day larval rearing period in the present study.

of histological changes in the intestine, providing deeper insights into the mechanisms driving temperature-dependent influences in aquaculture settings.

Methods

Induced spawning of broodstock

Ethical approval for this study was obtained from the University of Guilan, under approval number IR.GUILAN.REC.1400.050. Also, all animal-related procedures followed the ethical standards outlined in the ARRIVE guidelines and complied with Directive 2010/63/EU, ensuring the welfare of animals used in scientific research.

Eggs for this experiment were obtained from captive broodstock of strelet held at the Dr. Yousefpour Marine Fishes Restocking and Genetic Conservation Center (Siahkal, Guilan, Iran). Initially, 30 broodstocks were collected and oocyte maturity determined based on the position of germinal vesicle (GV)[3,37]. The average weight of the four females and two males was 1566.2 ± 0.3 g. The broodstocks were held in 800 L circular concrete tanks with a flow rate of 14.3 ± 0.2 L min^{-1}. The propagation process was conducted based on the GV index and water temperature, using intramuscular injection of LHRHa2 (pGlu-His-Trp-Ser-His-Gly-Ttp-Arg-Pro-Gly-NH2; San Shen Ningbo, Sheng, China). Females received two injections of 7 µg kg body weight BW^{-1}, while males were given a single injection of 3.5 µg kg BW^{-1} during the second injection phase for females[37].

After the collection of eggs and sperm, fertilization was performed using a semi-wet method[3]. To remove the stickness, the fertilized eggs were washed with a sodium hypochlorite solution for four minutes[38]. Following this, the eggs were transferred to Yushchenko incubators with a flow rate of 3.4 L min^{-1}.

Larvae maintenance

Six to eight hours after fertilization, when cell divisions were observed, the eggs were transported to the experimental unit. The tanks were filled with well water maintained at a temperature of 18 °C. Oxygen concentration was maintained at 8.1 ± 0.3 mg L^{-1}, and the photoperiod was set at 14 L:10 D. After hatching, larvae were distributed into nine square tanks (35 L) at density of 18 larvae L^{-1} and cultured for 64 days. Initially, the larvae were fed live newly hatched *Artemia urmiana* at 60% of BW starting 10 days post-fertilization (dpf). This was followed by the gradual introduction of frozen Chironomids (Biogrant, Tehran, Iran) at 30% BW[37,39]. Subsequently, the larvae were weaned from Chironomids to a formulated commercial pellet diet (Ata feed, Qazvin, Iran). Excess food and feces were regularly removed from the tanks, and 30% of the water was replaced daily to maintain optimal water quality.

Experimental design

For this study, 3 treatments in 3 replicates were used and water temperature was manipulated by aquarium glass heater (50 W, Aqua, Tehran, Iran). The design and setup of the experiment, which includes three different groups are detailed as below and illustrated in Table 3 and Fig. 1.

Group A: 8–32 dpf in ambient temperature.
Group B: 11–32 dpf at 21 °C.
Group C: 17–32 dpf at 27 °C.

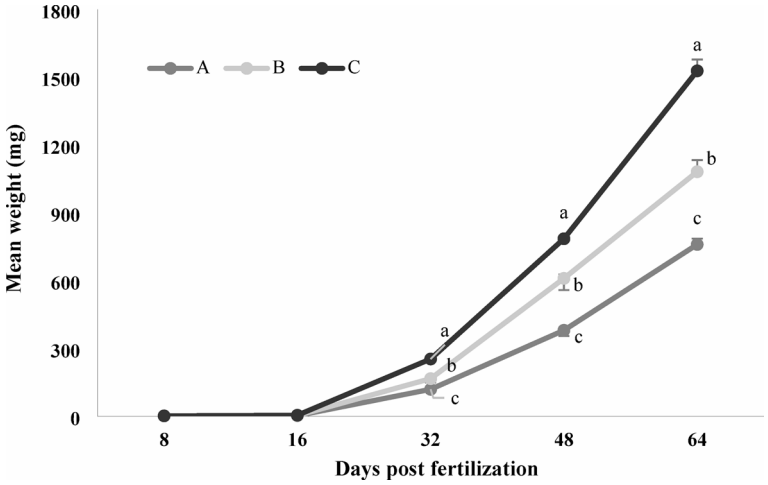

Fig. 2. Mean weight of sterlet (*Acipenser ruthenus*) larvae after 64 days thermal manipulation. Mean with different letters are significantly different for each group. A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C. Data are shown as mean ± standard error (SE) at $p < 0.05$.

The experimental period was considered from 8 to 64 dpf. Group A (control) from 8 to 64 was in ambient temperature (18 °C) but group B and C had 3 temperature windows. Group B experienced 3 temperature windows: 8–11 dpf at ambient temperature; 11–32 dpf at 21 °C; 32–64 dpf at ambient temperature. Also, group C experienced 3 temperature windows: 8–17 dpf at ambient temperature; 17–32 dpf at 27 °C; 32–64 dpf at ambient temperature. To prevent stress and thermal shock, the temperature gradually increased and decreased by 1°C per day to reach the respective temperatures. Also, water temperature was monitored three times daily to ensure precise temperature control.

Growth performance

At the end of the experiment, body weight and total length of sampled larvae were measured to the nearest 0.01 mg and 1 mm, respectively. Additionally, specific growth rate for weight and length (SGR_W and SGR_L), and daily growth rate (DGR) were calculated as follow[1]:

SGR_W (% day^{-1}) = 100 × (Ln final weight - Ln initial weigth) / days

SGR_L (% day^{-1}) = 100 × (Ln final length - Ln initial length) / days

DGR (%) = 100 × (final weight - initial weigth) / initial weight × days

Morphologic and meristic analyses

Larvae from each treatment were randomly sampled at 32 dpf ($n = 12$) and were euthanized using clove powder extract[4]. All samples were fixed in paraformaldehyde 4% (PFA) for 24 h, followed by methanol 100%. A double staining technique using Alcian blue 8GX (Sigma-Aldrich, Schnelldorf, Germany) and Alizarin red S (Sigma-Aldrich, Schnelldorf, Germany) was employed[40] to stain cartilage and mineralized bone, respectively. For staining, each container including the fish larvae were filled with 0.02% Alcian blue solution (containing 150 mM $MgCl_2$, ethanol 70%) for cartilage and 0.5% Alizarin red solution for bone and incubated overnight. All larvae were rinsed with distilled water to remove any residual stain. The samples were then placed in a bleaching solution containing KOH 1% (Sigma-Aldrich, Schnelldorf, Germany) and H_2O_2 1.5% for 20 min. Following this, the larvae were incubated overnight in a solution of glycerol 20% and KOH 0.25%, before being transferred to solution comtaining glycerol 50% and KOH 0.25%. Finally, the samples were stored in a solution of KOH 0.1% and glycerol 50% until further examination. Stained larvae were photographed using a stereo microscope (Olympus, Tokyo, Japan) equipped with a digital camera (VisiCam, Avantor, Radnor, USA). Additionally, head and body areas were measured using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Histological analysis

Larvae from each treatment were sampled at 32 and 64 dpf ($n = 9$). The larvae were then washed in distilled water to remove excess materials. Larvae were subsequently placed in PFA 4% solution and fixed overnight at 4 °C. The sampled larvae were washed in 100 mM sodium phosphate (pH = 7.4) and stored in methanol 100% for further examination[41]. Before tissue processing, larvae were decalcified using 4% EDTA (pH = 8) for a week to soften skeletal tissues. The samples were dehydrated through increasing concentration of ethanol (70, 96, 100%) in automatic tissue processor (Leica TP 1020, Lisbon, Portugal) and were embedded in paraffin using tissue embedding machine (KD-BM, KEDEE, Jinhua, China). The prepared sections (6 µm) using a microtome (RM 2145, Leica Microsystems, USA) were stained with hematoxylin and eosin solution (Sigma, Schnelldorf, Germany). Finally, the prepared slides were examined using a light microscope (Nikon E600, Tokyo, Japan) and photographed by camera at ×40 magnification. Histological sections of the tissues were analyzed using ImageJ software for quantitative analysis of morphological parameters including tubular muscularis, villi height, villi width and enterocyte height.

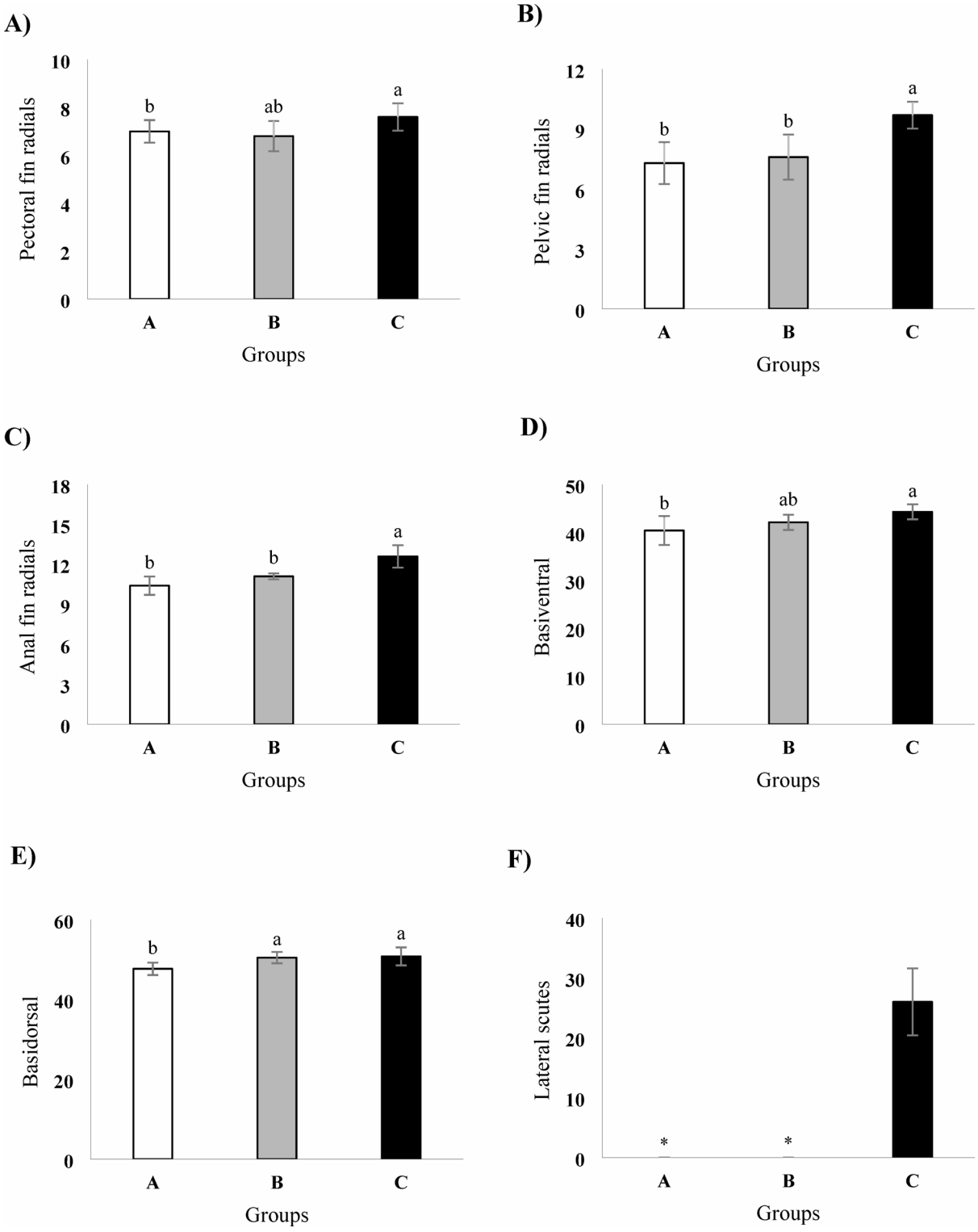

Fig. 3. Meristic trait development of sterlet (*Acipenser ruthenus*) after 32 days thermal manipulation. (**A**) number of pectoral fin radials (**B**) number of pelvic fin radials (**C**) number of anal fin radials (**D**) number of basiventral (**E**) number of basidorsal (**F**) number of lateral scutes. Mean with different letters are significantly different for each group. An asterisk (*) denotes groups with no observed values, indicating the absence of the measured trait. A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C. Data are shown as mean ± standard error (SE) at $p < 0.05$.

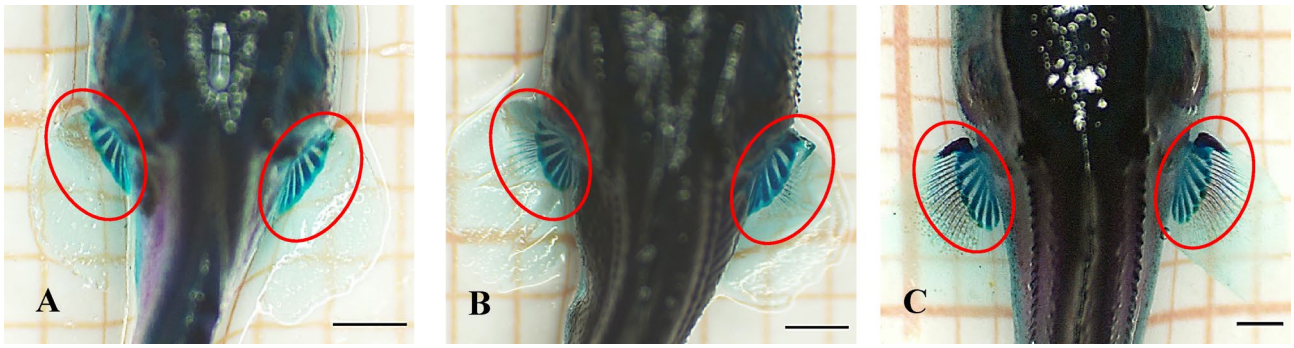

Fig. 4. Pectoral fin spin and distal radials of sterlet (*Acipenser ruthenus*) after 32 days thermal manipulation. (Scale: 1 mm). A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C.

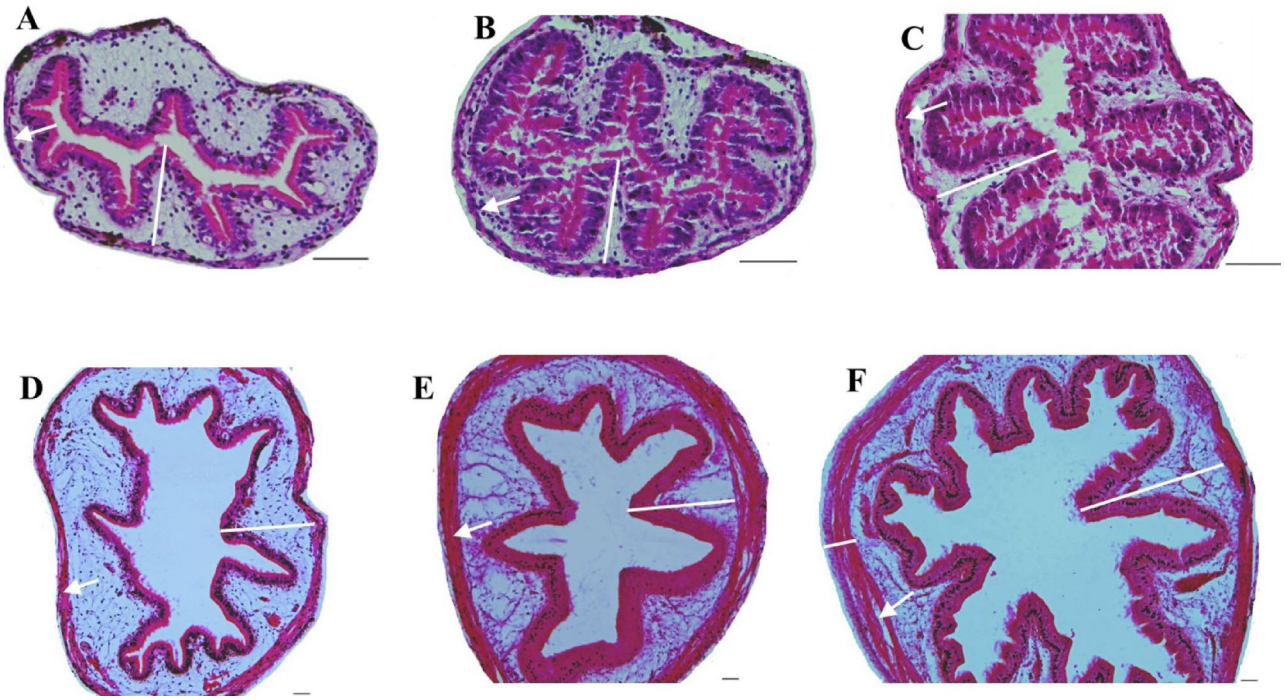

Fig. 5. Intestine morphology of sterlet (*Acipenser ruthenus*) after 32 and 64 days thermal manipulation (Scale: 50 µm). A-C: 32 dpf; A: 8–32 dpf in ambient temperature; B: 11–32 dpf at 21 °C; C: 17–32 dpf at 27 °C. D-F: 64 dpf; D: 8–32 dpf in ambient temperature; E: 11–32 dpf at 21 °C; F: 17–32 dpf at 27 °C. The figure includes straight lines to indicate villus height and arrows to highlight the tubular muscularis.

Statistical analysis

All statistical analyses were conducted using SPSS software (version 21, IBM, USA). The normality of data and homogeneity of variances were assessed using the Kolmogorov-Smirnov test and Levene's test, respectively. Based on these assumptions, one-way analysis of variance (ANOVA) was performed, with Tukey's post hoc test used to identify differences among the groups in growth, morphologic and meristic traits and intestine performance. Differences were considered statistically significant at $p < 0.05$.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Naghmeh Jafari Pastaki: Resource, Investigation, Methodology, Software, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. Hamed Abdollahpour: Resource, Formal analysis, Writing – review & editing. Bahram Falahatkar: Supervision, Project administration, Validation, Conceptualization, Writing – original draft. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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